PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

ANTHONY J. KINNEY
GARY M. FADER

CASE NO.: BB-1071-A

APPLN. NO.: 09/108,010

GROUP ART UNIT: 1638

FILED: JUNE 30, 1998

EXAMINER: E. MCELWAIN

FOR: SUPPRESSION OF SPECIFIC CLASSES OF SOYBEAN SEED PROTEIN GENES

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Gary Fader Pursuant to 37 CFR §1.132

I, Gary M. Fader, am a citizen of the United States of America, residing at 100 Woods Lane, Landenberg, PA 19350, United States of America, and I declare as follows:

1. I am one of the above-identified inventors named in this application. I am a graduate of the University of Toledo, Ohio with a B.A. degree granted in 1979 in Biology. I received an M.S. in Crop Physiology in 1981 and a Ph.D. in Crop Physiology in 1983 from Purdue University. I was a postdoctoral fellow at the Agronomy Department of the University of Wisconsin from 1983 to 1986. I have been employed by E. I. du Pont de Nemours and Company since 1986 directing and conducting research in developing herbicide resistant plant varieties and developing soybean lines with improved oil and protein qualities. I have built small-scale processing capabilities to produce oils and protein products for evaluation, developed small-scale functional tests predictive of performance in food applications, germplasm screening, manipulation of gene expression using molecular biology, and transformation and regeneration of plants.

2. I have reviewed the Office Action dated March 23, 2001. I am aware that this declaration is being submitted to address the concerns set forth on pages 2 and 3 of the Office Action that "the claims are broadly drawn to the use of an unspecified gene to produce the claimed plants and seeds with reduced levels of glycinin of β-conglycinin, yet the specification only teaches the use of one particular gene to produce said plants and seeds."

3. The rationale for combining the nucleic acid fragments of the invention clearly was disclosed in the specification. It was shown, for the first time, that two or more subunits of β-conglycinin could be suppressed using:

a) a truncated alpha subunit of β-conglycinin in sense orientation with respect to a promoter, or

b) β-conglycinin promoter and leader sequences directing the expression of sense FAD2, or

c) the entire alpha subunit coding region in anti sense orientation with respect to a promoter.

The specification also disclosed that expression of truncated glycinin subunits would suppress glycinin (all subunits).

4. Methods to prepare DNA fragments comprising truncated versions of the different glycinin subunits were set forth in the specification. The specification also described how to use these nucleic acid fragments to practice the invention.

5. The fragments corresponding to the glycinin Group I (G1) and Group II (G4) described in Example 4 of the specification (page 26 at line 3 through page 27 at line 31) were joined in a transcription unit under the control of the KTi promoter and used for bombardment into somatic embryo tissue. The transcription unit containing KTi promoter/G1/G4/KTi 3' end was cloned into the Bam HI site of pKS18HH. Plasmid pKS18HH is described in the application on page 15 at line 40 through page 16 at line 3 and is shown in the application's Figure 3. The plasmid used for bombardment contained:

a) the KTi promoter/G1/G4/KTi 3' end

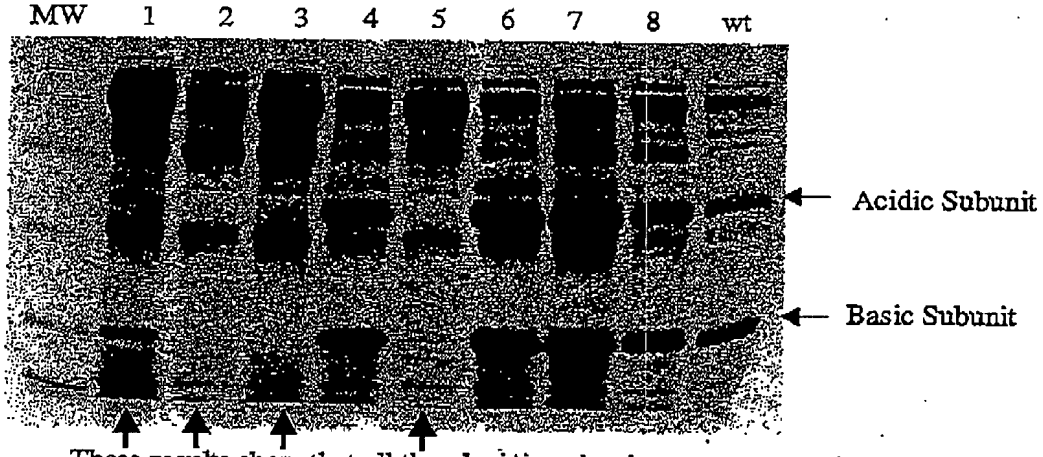
b) the T7 promoter/HPT/T7 Terminator Sequence

c) the CaMV 35S promoter/HPT/NOS 3' end

d) the vector sequences from pSP72 with the beta-lactamase coding region removed.

Bombardment and analyses were conducted as described in the specification on page 17 at line 10 through page 18 at line 37.

6. The results (an example of which is shown in the SDS PAGE gel of protein extracted from seeds of lines derived from regenerated plants) indicate that all the glycinin subunits are suppressed in some of the lines (indicated by arrows at the bottom of the gel).



These results show that all the glycinin subunits are suppressed when truncated forms of the G1 and G4 subunits are expressed in sense orientation under the control of the KTi promoter.

In summary, all of the elements of the claimed invention were provided in the patent application. The data presented in this declaration are consistent with the disclosure set forth in the specification.

Accordingly, one skilled in the art can take these elements, as discussed above, and practice the invention without undue experimentation.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Gary M. Fader

6-27-01
Date

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ANTHONY J. KINNEY
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EXAMINER: E. MCELWAIN

FOR: SUPPRESSION OF SPECIFIC CLASSES OF SOYBEAN SEED PROTEIN GENES

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Anthony J. Kinney Pursuant to 37 CFR §1.132

I am one of the above-identified inventors named in this application. I, Anthony John Kinney, am a citizen of the United Kingdom and am a permanent resident of the United States of America, residing at 609 Lore Avenue, Wilmington, Delaware 19809, and I declare as follows:

I received a B.Sc. in biology from the University of Sussex in 1980 and a D. Phil. in biochemistry and cell biology from Oxford University in 1985. I served as a research fellow in the Department of Food Science at Rutgers University, New Brunswick, N.J. 9/87-5/89. I have been employed at E. I. du Pont de Nemours and Company (DuPont) since June, 1989. I work as a technical leader for DuPont Crop Genetics and am presently working on expression of storage oil, protein and, carbohydrate genes. I have authored in excess of fifteen refereed articles in the field of biochemistry, with emphasis in the field of fatty acid and oil biosynthesis.

2. I have reviewed the Office Action dated March 23, 2001. I am aware that this declaration is being submitted to address the concerns set forth on pages 4 and 5 of the Office Action that "even though the product by process claims are limited and defined by a process, the determination of patentability is based on the product itself."

3. The results shown in the application indicate that the transgene responsible for the phenotype was integrated into a single locus. Example 2 of the application, in particular page 24 at lines 4 through 13, describes the isolation of transgenic soybean

sublines (G94-1, G94-19) with high oleic acid and suppressed β-conglycinin subunits derived from transformation event 260-05. The sublines are described as containing two copies of the plasmid pBS43 at a ***single*** locus, called the "transwitch locus" in example 2 and *locus A* in this declaration. *Locus* A is responsible for both the high oleic and the β-conglycinin null phenotype. Example 2 also states that R5 seeds derived from both of these sublines (G94-1, G94-19) have suppressed β-conglycinin subunits. (For clarity it should be noted that the terms "Transwitch locus" and "*locus A*" are used interchangebly herein.)

4. The Southern blot analyses presented below further analyze the transgenic seeds and plants and show that the phenotype is due to the presence of an insertion at a single locus. The experiments discussed herein were performed by me or others working under my guidance and direction or in coordination with the DuPont regulatory group.

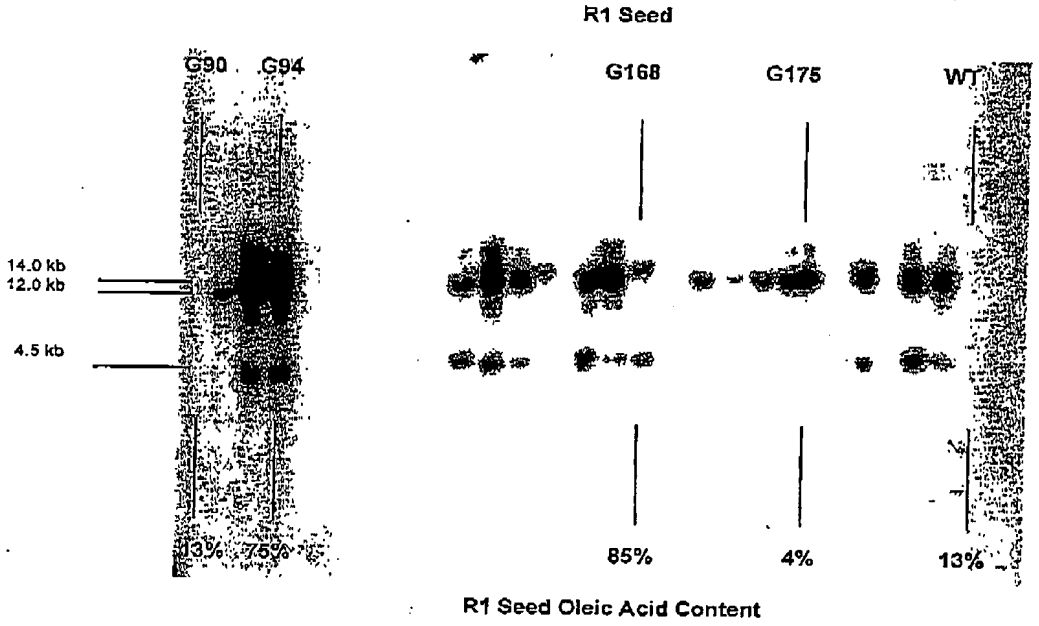
5. The nature of the insert at *locus A* ("transwitch locus") was initially determined by Southern blot analyses of DNA from leaves of R1 and R2 plants. Genomic DNA was digested with Bam HI and probed with the 3' region of phaseolin to detect the *GmFad 2-1* gene expression cassette. Bam HI cuts once in the plasmid and would be expected to result in one hybridising band for each copy of the plasmid inserted into the genome. The results of Southern blot analysis of DNA isolated from leaf tissue of event 260-05 R1 plants that were grown from chipped seeds analysed for fatty acid composition are shown below in Figure 1.

The DNA hybridisation pattern depicted in Figure 1 shows clearly that in the original transformation event the *GmFad 2-1* construct was integrated at two different loci in the soybean genome. At *locus A* the *GmFad 2-1* construct silenced the endogenous *GmFad 2-1* gene, resulting in seeds with an oleic acid content generally above 80%. *Locus A* contained two copies of the *GmFad 2-1* expression cassette as indicated by the two hybridising fragments of 14.0 kb and 4.5 kb. The second locus (*locus B*) contained a copy of *GmFad 2-1* that was over-expressing thus decreasing oleic acid levels to around 4%. *Locus B* contained only one copy of the *GmFad 2-1* expression cassette as noted by the single hybridising fragment of 12.0 kb.

Since G94 contained both loci in the R1 plant an additional round of selection was necessary on the segregating R2 plants to isolate plants containing *locus A* and not *locus B*. Southern blot analysis on genomic DNA isolated from leaf tissue of R2 plants grown from G94 R2 seed using Bam HI digestion and the phaseolin 3' probe identified two sublines, G94-1 and G94-19, that contained *locus A* but not *locus B* since *locus B* had been removed by segregation. *Locus B* was not further

characterized. Figure 2 shows Southern blot analysis of R1 and R2 leaf tissue originating from 260-05 G94 R1 seed. The genomic DNA was digested with Bam HI and probed with the phaseolin 3' probe to detect the integration of the GmFad 2-1 construct.

Figure 1



Additional Southern blot analyses using DNA from the leaves of R6 plants, using multiple restriction enzymes, confirmed that G94-1 and G94-19 contained a single transgenic locus (*locus A*, the "Transwitch locus"). Figure 3 shows the results of Southern blot analyses of DNA isolated from R6 leaf tissue of G94-1 and G94-19, of a sister line from the same event (G168), and of control elite soybean line A2396. In this figure DNA was digested with either Bam HI, Bsp HI, Hind III, or Sst I and hybridized with the phaseolin 3' probe. The Bam HI pattern is identical to the R2 plants.

7. The results mentioned in the application and expanded upon above indicate that suppression of β-conglycinin subunits is caused by an insertion in a single locus of the transgenic plant.

Figure 2

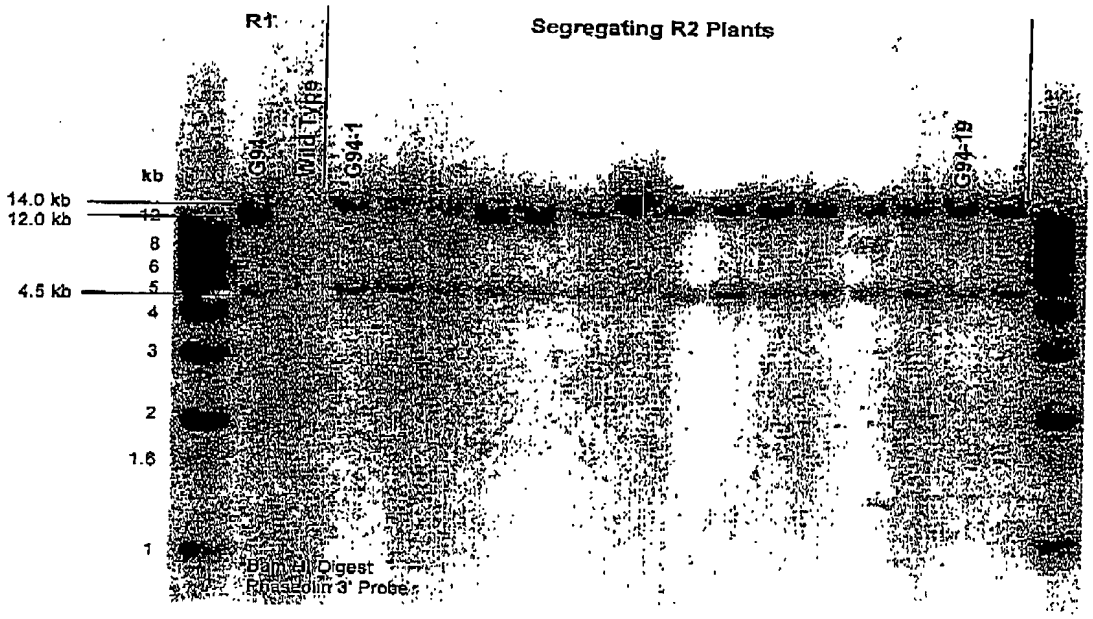
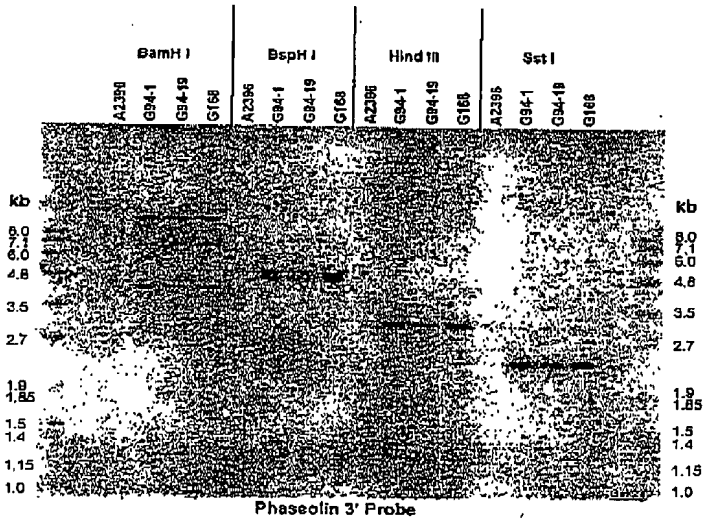
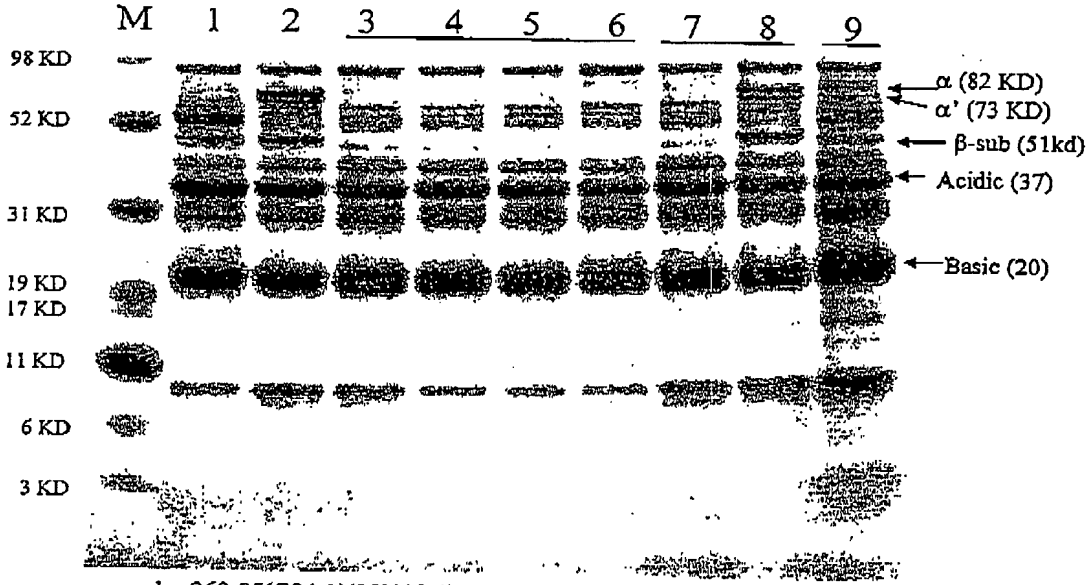


Figure 3



8. Furthermore, analysis, as described in Example 3, of other transgenic soybean lines containing exactly the same α'-subunit promoter sequence of the construct in Example 2 indicates that R1 seeds of these events lack the α, α', and sometimes β subunits of β-conglycinin. Figure 4 shows a picture of a Coomassie brilliant blue R-stained SDS polyacrylamide gel where 20 μg total protein was loaded per lane. The origin of the material loaded on each lane is indicated below the picture of the gel.Figure 4

Figure 4

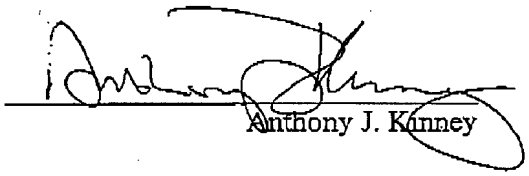


1. 260-05(G94-1)(85%18:1)
2. JACK (20%)
3. 2308-22-16-H5 (80%)
4. 2308-22-3-14 (80%)
5. 2308-23-5-H1 (72%)
6. 2308-23-6-H13 (72%)
7. 2323-31-1-H34(82%)
8. 2323-31-10-9 (82%)
9. 2335-26-14-11 (80%)
10. 2335-26-2-8 (80%)

In summary, all of the elements of the claimed invention were provided in the patent application. The data presented in this declaration are consistent with the disclosure set forth in the specification.

Accordingly, one skilled in the art can take these elements, as discussed above, and practice the invention without undue experimentation.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Anthony J. Kinney

29 JUNE 2001

Date